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Research Article

The Phenotypic Detection of Carbapenem Resistant Organisms in Orthopaedic Wound Infections in Ile-Ife, Nigeria

Alaka OO[1,4], Orimolade EA[2,4], Ojo OO[3] and Onipede AO[1,4]*

[1]*Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria*

[2]*Department of Orthopaedics and Traumatology, Obafemi Awolowo University, Ile-Ife, Nigeria*

[3]*Department of Biological Sciences, Albany State University, Albany GA, USA*

[4]*Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria*

***Corresponding Author:** Onipede AO, Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. **E-mail:** aonipede@oauife.edu.ng

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Abstract

Introduction: We conducted the phenotypic detection of carbapenem resistant organisms in orthopaedic wound infections (OWI) in Ile-Ife, Osun State, Nigeria and their antibiotic susceptibility pattern, to evaluate the occurrence and characteristics of resistance.

Methodology: A cross sectional study was conducted from January to June 2015, to determine the main pathogens causing OWI and their antibiotic suceptibility patterns. Sample size was 180, collected from 153 patients. Bacterial isolates were identified after routine culture using MICROBACT kits (Oxoid, England) and antibiotic susceptibility was evaluated by the Kirby-Bauer method. Screening and phenotypic confirmation of carbapenemase and metallo-beta lactamase (MBL) production were done by Modified Hodges Test and Disk potentiation method respectively as prescribed by the Clinical and Laboratory Standards Institute (CLSI).

Results: Of the 180 specimens processed, 123 (68.33%) yielded growth of 162 isolates, 92 (56.79%) were Gram negative bacilli (GNB). *Staphylococcus aureus* 30.86% (n=50) was the predominant isolate out of the total organisms, followed by *Pseudomonas aeruginosa* 22.84% (n=37). GNB resistance to imipenem was 7.61%, and for Gram Positive Cocci (GPC) was 4.92%, while GNB resistance to meropenem was 14.13% and for GPC isolates 16.39%. Carbapenemase production was observed in 8.71% of GNB isolates with highest prevalence in *Pseudomonas aeruginosa* (5.44%) isolates while MBL production was observed in 5.44% of total GNB isolates.

Conclusions: The study showed *Pseudomonas aeruginosa* is the predominant GNB while *Staphyloccocus species* is the predominant GPC found in OWI in this environment. Carbapenem resistance was observed both in GPC and GNB. Carbapenemase production including MBLs was highest in *Pseudomonas aeruginosa*. The occurrence and possible spread of carbapenemases especially MBL among GNBs has been established in this environment.

Keywords: Carbapenemase Detection; Antibiotics Resistance; Orthopaedic Infections

Introduction

Orthopaedic wound infections may result from trauma, osteomyelitis or post-operative complication in form of surgical site infection (SSI). This leads to increased antibiotic use, prolonged hospital stay, increased cost of treatment, repeated debridements, prolong rehabilitation, morbidity and mortality [1].

The common organisms encountered in post-operative orthopaedic wound infections are *Staphylococcus aureus, Coagulase-negative Staphylococci, Enterococci, Proteus, Pseudomonas* and *Escherichia coli* just to mention a few [2,3].

Although the incidence of SSI after orthopaedic surgery has been reducing due to modern theatre facilities, aseptic measures and the institution of antibiotics Stewardship programmes in developed countries but its prevalence is still high in developing countries and it is further complicated by the presence of antibiotic resistant organisms such as extended spectrum Beta Lactamase (ESBL) producing Gram negative bacilli (GNB) as well as carbapenemase producing GNB [4]. Antibiotics stewardship programmes are still at the infancy stage in developing countries [1].

Carbapenems are regarded as the antibiotics of last resort, which makes the situation grave for patients with orthopaedic

wound infections in developing countries. While there are data from different countries on the prevalence of carbapenem resistant organisms, there is limited data on carbapenemase producing GNB in our setting, especially among orthopaedic patients.

We therefore undertook to investgate the occurrence and characteristics of carbapenems resistance among the isolates implicated in OWI, and their resistance to other classes of antibiotics were determined as well. We also related our findings to parameters such as type of bone affected, type of isolate, patient age and sex, and the duration of hospital stay for significance.

Materials and Methods

Study design and sample collection

This was a prospective cross-sectional study. A total of 180 samples were collected (wound swabs; 168, bone aspirates/biopsies; 12) from 153 patients clinically suspected or diagnosed with orthopaedic wound infections of different age groups and sexes attending orthopaedic clinic, as well as those admitted to the ward at Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife (OAUTHC) were enrolled in this study from January 2015 to June 2015. Specimens were collected after obtaining informed consent from the patients and patient's parent or guardian if a minor as applicable. Samples were collected from patients of all ages with orthopaedic diagnosis (in-patients and out-patients). A purposeful sampling method comprising of diagnosed or clinically suspected cases of OWI was adopted.

Ethical approval

Ethical approval was granted by the Ethics and Research Committee of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife.

Bacterial isolation and identification

The wound swab specimens and bone aspirates collected were inoculated on blood agar and MacConkey agar plates (Oxoid, England) and were incubated at 37^{0}C for 24 hours. The wound aspirates/biopsies were first incubated in tryptone soya broth (MAST Diagnostics, Merseyside, UK) at 37°C for 18 hours before sub-culturing to horse blood agar and MacConkey agar plates.

Identification of bacterial isolates was done using colony and microscopic morphology as described by Cheesbrough [5]. Conventional biochemical tests were performed using the GNB 24E and 12S Microbact Kit (Oxoid, England) for Gram-negative bacilli and Gram positive cocci respectively. Pure cultures in broth were stored in sterile glycerol at -20°C as stock and on tryptic soy agar (Biolab, Hungary) slants for further studies.

Antibiotic susceptibility testing of isolates

The antibiotic susceptibility testing was performed by the Kirby-Bauer Disc Diffusion method. [6] The inoculum was prepared from an overnight culture in tryptic soy broth.

From this culture, a 0.5 (625 nm) McFarland standard suspension was prepared in sterile normal saline, 0.85% NaCl (w/v) using a colorimeter and subsequently plated on the entire surface of a dry sterile Mueller Hinton agar plate (Oxoid, England) as previously reported [4].

The antibiotics used in testing included: IMI- Imipenem (10 µg), MEM-Meropenem (10 µg), CAZ- Ceftazidime (30 µg), CXM-Cefuroxime (30 µg), GM- Gentamicin (30 µg), OFX- Ofloxacin (5 µg), AUG- Amoxicilin (20 µg) with clavulanic acid (10 µg), CFM-Cefixime (5 µg), CIP- Ciprofloxacin (5 µg), ERY- Erythromycin (5 µg), CXC-Cloxacillin (5 µg) whether it is Gram-positive or Gram-negative on discs. All plates were incubated at 37°C for 24-36 h. The diameters of zones of inhibition were measured to the nearest millimeter using a ruler. Approved CLSI [7] susceptibility zone diameter interpretative standards were used.

Detection of carbapenemase production

Screening for carbapenemase production was done as prescribed by the Clinical and Laboratory Standard Institute [7]. Meropenem and Imipenem discs (10 µg, Mast, UK) were used. The antibiotic discs were placed on the surface of inoculated Mueller Hinton Agar (MHA) plates using sterile forceps. The discs were placed about 30 mm apart and the plates were incubated for 24 hours at 37^{0}C after which zones of inhibitions were read. Isolates that showed a zone of inhibition ≤ 21mm in diameter for Meropenem or ≤ 23mm in diameter for Imipenem were considered as suspected carbapenemase producers and were subjected to confirmatory test by the Modified Hodges Test (MHT).

Phenotypic confirmation of carbapenemases (Modified Hodges Test)

An overnight culture suspension of *Escherichia coli* ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a Mueller-Hinton agar. After drying, 10 µg Imipenem disk (Mast, UK) was placed at the center of the plate and the test strain was streaked from the edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight at 37^{0}C. The presence of a 'cloverleaf shaped' zone of inhibition due to carbapenemase production by the test strain was considered as positive [7].

Detection of metallo-beta lactamase (MBL) producing isolates by disk potentiation method

Confirmation for the detection of MBL was done by disc potentiation test with Ethylenediamine Tetraacetic Acid (EDTA)-impregnated imipenem discs. Two 10µg imipenem discs were placed on the plate and 5µl of EDTA solution was added to one of the disc. The inhibition zones of the imipenem and imipenem-EDTA discs was compared after 24 hours of incubation at 37^0C. An increase in the zone size of at least 7 mm around the imipenem-EDTA disc was recorded as MBL positive. EDTA was tested alone on the isolate(s) before incorporation into the antibiotic disk (s) in order to ensure that it did not inhibit the test organism and cause a false positive result [7].

Quality control

All prepared media were checked for sterility for 24 h. *E. coli* ATCC 25922 was used as a quality control strain for antibacterial susceptibility testing. The *E. coli* strain was also used as a negative control in the screening and phenotypic confirmatory tests of carbapenemase-producing Gram-negative rods.

Data analysis

Data are presented as frequencies and/or percentages. Statistical analysis was performed using the chi-square ($\chi 2$) Fischer's exact test in PAST software package by Hammer*., et al.* [8]. A *p* (predictive) value of <0.05 was considered as significant association between the variables tested.

Results

One hundred and eighty (180) samples were collected from 153 patients, age range was 6 – 85 years. There were 111 males and 42 Females in the study: M: F (2.6:1), the age and sex distribution are as stated in (Table 1). Diagnosis was stratified as fractures - 103 (Open 72, Closed 31), osteomyelitis - 23, tumours -5 and others - 22. The distribution of orthopaedic wound sites in relation to cause of injury is stated in (Table 2). Of the 180 orthopaedic wound sites sampled in this study, 142 (78.9%) were from lower limb especially tibia and fibular infection primarily due to fracture sustained from Road Traffic Accident (RTA) on motorcycle and this single factor accounted for 66.10% of RTA cases. The age bracket 21-30 years had the highest rate of wound infection observed from RTA. One incidence of mortality was recorded during the study from complications arising from RTA (Table 3).

Table 1: Age distribution of Patients

S/N	Age Group in Yr.	Male (%)	Female (%)	Total (%)
1	0 to 10	8 (5.23)	3 (1.96)	11 (7.19)
2	11 to 20	10 (6.54)	2 (1.31)	12 (7.84)
3	21 to 30	35 (22.88)	6 (3.92)	41 (26.82)
4	31 to 40	18 (11.76)	11 (7.19)	29 (18.95)
5	41 to 50	12 (7.85)	6 (3.92)	18 (11.76)
6	51 to 60	14 (9.15)	4 (2.61)	18 (11.76)
7	61 to 70	6 (3.92)	6 (3.92)	12 (7.84)
8	70 and above	8 (5.23)	4 (2.61)	12 (7.84)
9	Total (%)	111 (72.56)	42 (27.44)	153 (100)

Table 2: Distribution of Orthopaedic wound site in relation to cause of wound or injury.

Cause	Number	Percentage (%)
Osteomyelitis	24	13.33
Gun shot	4	2.22
Domestic accident	7	3.89
Occupational/industrial trauma	6	3.33
Diabetic foot	14	7.78
Atherosclerotic gangrene	1	0.56
Trauma during sport activities	2	1.11
Septic arthritis	3	1.67
Fire accident	1	0.56
Road traffic accident with motorcycle	78	43.33
Other form of Road traffic accident	40	22.33
Total	180	100

Table 3: Distribution of wound site.

Location of Wound	Number Examined	Number infected	Percentage Infected
Lower limb	142	100	70.4
Upper limb	31	19	61.3
Head	6	3	50
Vertebral column	1	1	100
Total	180	123	68.3

Sixty-eight percent (123) of the samples were culture positive and 32% were culture-negative. With respect to Gram morphology, 57% (70) were Gram-negative while (53) 43% were Gram-positive. From 123 culture positive samples, 86 (69.9%) samples yielded a single organism on culture and 37 (30.1%) samples yielded mixed organisms. In all, there were 162 isolates altogether, 92 (56.8%) isolates were Gram negative bacilli whereas, Gram positive cocci bacteria were 61 (37.7%) of the isolates, Gram positive bacilli bacteria accounted for 5 (3.08%) and finally *Candida species* were 4 (2.47%) isolates. From the culture positive samples (123), 43 (34.95%) were from patients who have had surgical intervention on account of RTA, from this group, 29 (67.44%) were from patients who had stayed 48 hours or more on admission preoperatively before surgical intervention.

Staphylococcus aureus was the most prevalent species of organism isolated accounting for 30.9% (n=50) of the total isolated organisms followed by *Pseudomonas aeruginosa* which accounted for 22.8% (n=37) of the isolates (Table 4). Other isolates recovered are, *Klebsiella pneumoniae* 16 (9.88%), *Staphylococcus epidermidis* and *Klebsiella oxytoca* 8 (4.94%) each, *Enterobacter agglomerans* was 6 (3.70%) and *Proteus mirabilis* 5 (3.09%) of the total isolates. Other bacteria species isolated and there frequencies were presented in table 4.

Ninety-two Gram negative bacilli (GNB) isolates were tested for their antibiotics sensitivity profile, From these, 43 were *Pseudomonas species*, from which 38 (88.4%) were sensitive to imipenem, 32 (74.4%), sensitive to meropenem, while 2 (4.6%) were of intermediate resistance to meropenem, 28 (65.1%) were sensitive to ceftazidime, and 3 (7.0%) were sensitive to cefuroxime. Other antibiotics tested against Pseudomonas and other GNB are as indicated in (Table 5).

For *Klebsiella species*, of the twenty four (24) isolates, 23 (95.8%) were sensitive to Imipenem and, 23 (95.8%) were sensitive to meropenem, 16 (66.7%) were sensitive to ceftazidime while 2 (8.3%) were of intermediate resistance to ceftazidime, and 12 (50%) were sensitive to cefuroxime (Table 5).

For the six *Proteus species* isolates, 5 (83.3%) were sensitive to ceftazidime, while 1 (16.7%) was of intermediate resistance to ceftazidime, 5 (83.33%) were sensitive to cefuroxime while 1 (16.7%) was of intermediate sensitivity to cefuroxime. All *Proteus species* isolated were sensitive to both imipenem and meropenem while all were resistant to gentamicin. Other antibiotics tested against *Proteus spp* are as indicated in (Table 5).

Table 4: Bacteria species isolated and their frequencies.

Organisms	Number	Percentage
Pseudomonas aeruginosa	37	22.84
Pseudomonas flourescens	1	0.62
Pseudomonas putida	3	1.85
Pseudomonas stutzeni	2	1.24
Providencia stuatii	3	1.85
Providencia rettgeri	1	0.62
Proteus mirabilis	5	3.09
Proteus vulgaris	1	0.62
Klebsiella pneumoniae	16	9.88
Klebsiella oxytoca	8	4.94
Acinetobacter lwoffi	1	0.62
Alcaligenes feacalis	2	1.24
Citrobacter freundii	2	1.24
Burkholderia capacia	1	0.62
Escherichia coli	1	0.62
Enterobacter agglomerans	6	3.7
Serratia marcescens	2	1.24
Staphylococcus aureus	50	30.86
Staphylococcus chromogenes	1	0.62
Staphylococcus hominis	1	0.62
Staphylococcus simulans	1	0.62
Staphylococcus epidermidis	8	4.94
Candida species	4	2.47
Gram positive bacilli	5	3.09
Total	162	100

Of *Enterobacter agglomerans* (6) isolates six (6) in nuber, 2 (33.3%) were sensitive to ceftazidime, while 2 (33.3%) were of intermediate resistance to ceftazidime, and 2 (33.3%) were sensitive to cefuroxime. All *Enterobacter agglomerans* isolated were sensitive to both imipenem and meropenem. Other antibiotics tested against Enterobacter agglomerans are as indicated in (Table 5).

All Providencia species (4) isolated were sensitive to imipenem, meropenem, ceftazidime, cefuroxime, and cefixime, while for Citrobacter species (2) isolates, both were sensitive to imipenem and meropenem but were resistant to amoxicillin-clavulanic acid, cefixme and ciprofloxacin (Table 5).

The antibiotics sensitivity of other Gram negative isolates such as *Alcaligenes faecalis, Serratia marcescens, Acinetobacter lwoffi, Burkholderia capacia* and *Escherichia coli* are as indicated in (Table 5).

Table 5: Antibiotic sensitivity pattern of Gram-negative organisms.

Organisms	IMI (%)	MEM (%)	CAZ (%)	CXM (%)	GM (%)	OFX (%)	AUG (%)	CFM (%)	CIP (%)	Total
Pseudomonas spp	38 (88.37)	32 (74.41)	28 (65.12)	3 (6.98)	10 (23.26)	8 (18.60)	22 (51.16)	3 (6.98)	7 (16.28)	43
Klebsiella species	23 (95.83)	23 (95.83)	16 (66.67	12 (50)	9 (37.50)	9 (37.50)	3 (12.5)	8 (33.33)	6 (25)	24
Proteus species	6 (100)	6 (100)	5 (83.33)	5 (83.33)	6 (0)	2 (33.33)	4 (66.67)	4 (66.67)	1 (16.67)	6
A. iwoffi	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1
P. species	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	2 (50)	1 (25)	4 (100)	2 (50)	4
Citrobacter spp	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	2
B. capacia	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
Escherichia coli	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
E. agglomerans	6 (100)	6 (100)	2 (33.33)	2 (33.33)	2 (33.33)	4 (66.67)	1 (16.67)	0 (0)	1 (16.67)	6
A. feacalis	1 (50)	1 (50)	2 (100)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	2
S. marcescens	2 (100)	2 (100)	2 (100)	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	1 (50)	2
Total	85 (92.39)	79 (85.87)	61 (66.30)	27 (29.35)	33 (35.87)	28 (30.44)	31 (33.70)	19 (20.65)	19 (20.65)	92
P value	0.012	0.224	0.242	0.242	0.308	0.166	0.259	0.238	0.05	
	SG	NS	NS	NS	NS	NS	NS	NS	SG	

IMI: Imipenem (10 µg); MEM: Meropenem (10 µg); CAZ: Ceftazidime (30 µg); CXM: Cefuroxime (30 µg); GM: Gentamicin (30 µg); OFX: Ofloxacin (5 µg); AUG: Amoxicilin (20 µg) with clavulanic acid (10 µg); CFM: Cefixime (5 µg); CIP: Ciprofloxacin (5 µg).

The anitibiotic sensitivity of Gram positive isolates (61), are as follows: *Staphylococcus aureus* (50), from which, 49 (98%) were sensitive to imipenem, 43 (86%) to meropenem, 24 (48%) to ceftazidime while 6 (12%) were of intermediate resistance to ceftazidime, 39 (78%) were sensitive to cefuroxime, while 4 (8%) had intermediate resistance to cefuroxime, 18 (36%) were sensitive to ofloxacin, while 11 (22%) were of intermediate resistance to ofloxacin, and 25 (50%) were sensitive to augmentin. Other antibiotics tested against Gram positive isolates are as indicated in (Table 6).

Of the *Staphylococcus epidermidis* isolates (8 in number), from this, 6 (75%) were sensitive to imipenem, 3 (37.50%) to meropenem while 1 (12.50%) was of intermediate resistance to meropenem, 2(25%) were sensitive to ceftazidime, 4(50%) to cefuroxime, 3 (37.50%) to ofloxacin while 1 (12.50%) was of intermediate resistance to ofloxacin, and 4 (50%) were sensitive to augmentin (Table 6).

The antibiotics sesnsitivity of *Staphylococcus chromogene* (1), *Staphylococcus homonis*(1) and *Staphylococcus simulans* (1) are as indicated in (Table 6), they are all sensitive to imipenem and meropenem.

Table 6: Antibiotic sensitivity pattern of Gram-positive organisms.

Organisms	IMI (%)	MEM (%)	CAZ (%)	CXM (%)	OFX (%)	AUG (%)	ERY (%)	CXC (%)	Total
S. aureus	49 (98)	43 (86)	24 (48)	39 (78)	18 (36)	25 (50)	7 (14)	7 (74)	50
S. epidermidis	6 (75)	3 (37.50)	2 (25)	4 (50)	3 (37.50)	4 (50)	1 (12.50)	5 (62.50)	8
S. chromogenes	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1
S. hominis	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1
S. simulans	1 (100)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1
Total	58 (95.08)	49 (80.33)	26 (42.62)	45 (73.77)	22 (36.07)	31 (50.81)	9 (14.75)	44 (72.13)	61
P value	0.04	0.04	0.157	0.092	0.199	0.092	0.223	0.092	
	SG	SG	NG	NG	NG	NG	NG	NG	

IMI: Imipenem (10 µg); MEM: Meropenem (10 µg); CAZ- Ceftazidime (30 µg), CXM: Cefuroxime (30 µg); OFX: Ofloxacin (5 µg); AUG: Amoxicillin (20 µg) with clavulanic acid (10 µg); ERY: Erythromycin (5 µg); CXC: Cloxacillin (5 µg).

From the foregoing, Carbapenem resistance in all was 7.61% and 14.61% among GNBs for Imipenem and Meropenem respectively, while the resistance rate among Gram positive cocci (GPC) isolates for Imipenem and Meropenem were 4.92% and 16.39% respectively. Carbapenemase production was observed in 8.71% of GNB isolates with highest prevalence in *Pseudomonas aeruginosa* isolates while Metallobeta-lactamase production was observed in 5.44% of total GNB isolates. *Pseudomonas aeruginosa* alone accounted for 3 (60%) of the 5 (100%) MBL producing isolates.

Discussion

This study established the presence of Carbapenem resistant organisms in orthopaedic wounds in Ile-Ife, Nigeria. Carbapenemase production was observed in 8.7% of GNB isolates with highest prevalence in *Pseudomonas aeruginosa* isolates. The prevalence of carbapenemase-producing isolates in hospital settings ranged from 2.3% to 67.7% in North Africa and from 9% to 60% in sub-Saharan Africa as reported by Rendani., *et al*. [9] in a systematic review of the spread of carbapenemase-producing bacteria in Africa. The prevalence rate found in this study is less than the lower limit in the systematic review but is still of considerable concern because Carbapenems are regarded as the last line of defense against ever more prevalent Multi Drug Resistant Gram negative bacilli (MDR-GNB). Specifically, MBL production observed in 5.4% of total GNB isolates in this study is low compared to 25.3% of MBL reported for Gram-negative isolates of nosocomial pneumonia [10] and similar studies from other settings ranging between 5.75% to 51%. Also, the incidence of Carbapenemase producing isolates varies from one region to another, and as well as from time to time across yearly seasons. The present low rate of MBL in this environment mirrors the early stages of Carbapenem use.

In this study, trauma due to RTA was the major risk factor for wound infection, followed by osteomyelitis. We observed a preponderance of male with orthopeadic wound infection. This is not surprising in this environment due to RTA consequent upon the use of motorcycle as a major means of transportation and commuting in developing countries. Sixty-seven percent of the patients had fractures. Orthopaedic wound infection is a fairly common event globally with a preponderance of males affected. The incidence of open fracture wounds was previously reported in this environment to be 78.7% in male patients and 21.3% in female patients [11], so the epidemiology as not changed much.

A variety of organisms have been isolated as the aetiology of orthopaedic wound infections arising from trauma, osteomyelitis or post-operative complication in form of surgical site infection (SSI). With open fractures, the infection rate was 45.8%. Gram-positive cocci and Gram-negative rods were isolated [12]. Organisms commonly encountered include *Staphylococcus aureus,* Coagulase-negative Staphylococci, Enterococci, Proteus, Pseudomonas and *Escherichia coli* [1]. In this study, the types of organisms isolated were not different from what have been reported in the literature generally with Gram-negative bacteria being the major etiologies of wound infections [2,4,13,14]. In this present study *Staphyloccocus species* (30.9%) was the predominant Gram positive cocci found in orthopaedic wound infection in Ile-Ife while *Pseudomonas aeruginosa* (22.8%) were the predominant Gram negative bacilli (GNB). This finding is in agreement with a previous study of Ojo., *et al*. [15] from this environment but differed with respect to the predominant GNB from a 2009 study [11] which indicated *Escherichia coli.* However, in another study from this hospital, the predominant bacteria were *Klebsiella spp*. and *E. Coli* at that time [4]. This might be due to the changing pattern of bacteria flora of orthopaedic wound infections in this environment or the different populations studied. Furthermore, infection of wounds by microorganisms is most often associated with prolonged hospital stay, comorbidities and the premorbid state of the patients coupled with the attendant risk of acquisition of multiple resistant organisms from medical devices. These are factors that can drive the changing pattern observed.

We have reported previously, the incidence of extended spectrum beta lactamase in orthopaedic wound infection in this same setting [4]. In this study, both Gram-negative and Gram-positive organisms are predominantly sensitive to the carbapenems but less so to the third generation cephalosporin indicating the presence of extended beta lactamase activity in this organism. However, several years after the previous publication, the picture of multiply resistant organisms in orthopaedic infection has assumed a proportion of grave concern considering that they are regarded as antibiotics of last resort. Several risk factors have been identified as responsible for development of resistance in the clinical settings such as increased antibiotic use and prolonged hospital stay leading to increased cost of treatment, repeated debridement, prolong rehabilitation, and more importantly, the absence of any policy on antibiotic stewardship.

Carbapenemase-producing Enterobacteriaceae (CPE) are now reported globally leading to limitations of treatment options [16]. This study established the presence of CPE in orthopaedic wound infection in our clinical setting, although the introduction of carbapenem is relatively recent in this environment, yet carbapenemase

production in Gram negative isolates in the present study was 8.7% compared to a report from the northern part of Nigeria in which CPE was 13.3% [17] in non-orthopaedic subjects whereas a study from India recorded all study isolates (n=111) phenotypically tested were positive for Carbapenemse [16]. The authors went further to identify the presence of carbapenemase-encoding genes in 67 isolates of the total. Our study did not proceed further to identify the presence of carbapenemase-encoding genes due to limited resources which we consider to be a limitation. However, authors from different parts of the world who similarly conducted phenotypic studies, have reported varying resistance rates of CPE from 5.7% to 51% [18-21].

Though there is a dearth of information on MBL production in clinical isolates in Nigeria. Metallobeta-lactamase production observed in this study of 5.44% (n=5) of total GNB isolates, is also of concern. Other studies from different parts of Nigeria, for example one from the south-west, (4) 4.1% out of 97 *P. aeruginosa* isolates [22]; southeastern 13.1% showed MBL production overall. Attention has been drawn to the ascendancy of metallo-beta-lactamases within the clinical sector with some reports indicating that nearly 30% of imipenem-resistant *Pseudomonas aeruginosa* strains possess a metallo-beta-lactamase. Their clinical significance is further embellished by their ability to hydrolyze all beta-lactams and by the fact that there is currently no clinical inhibitor, nor is there likely to be for the foreseeable future [23].

Conclusion

In conclusion, our study established the presence of Carbapenemase producing isolates in orthopaedic wound infection in our clinical setting. Though still relatively low compared to other reported settings, there is a required need for increased institutional surveillance for antibiotics resistance particularly, carbapenemases, and as well as the establishment of Antibiotic stewardship measures to prevent rising incidence of antibiotics resistance, by ensuring prudent use of antibiotics generally. Furthermore, the present study has highlighted the changing pattern of organisms isolated from orthopaedic wound infections and their antibiotic sensitivity pattern in this environment with the most common causative agents of orthopaedic wound infection being *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Klebsiella oxytoca.*

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Conflict of Interest

No conflict of interest exists.

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